LOCAL DEPILATORY ACTION OF UNSATURATED COMPOUNDS

The Effect of Human Sebum on Hair Growth*

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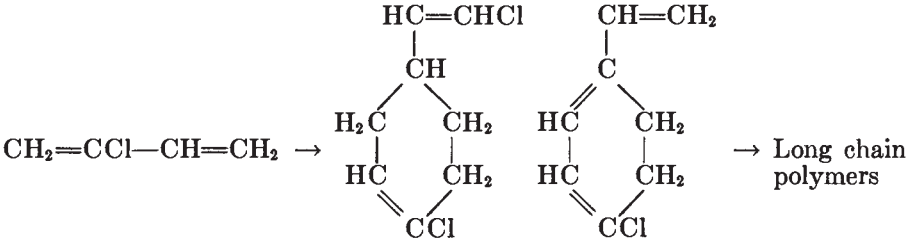
This paper deals with a group of compounds characterized by a new pharmacologic action: complete temporary local depilation. Topical application of these substances to the skin of laboratory mammals results in reversible total hair loss, i.e. shedding of the hairs with the roots, leading to alopecia.

The first depilatory agents of this group were discovered accidentally in the Czech (1), American (2, 3) and Swedish (4) synthetic rubber industry, where it was observed that many of the workers who were engaged in the manufacture of neoprene rubber lost their hair. This hair loss had several characteristic features: 1. It involved only the scalp hair. 2. The alopecia was complete, that is, the hair came out by the root. 3. The condition was always reversible. Some workers lost their hair as frequently as seven times, but this loss was never permanent. 4. The loss of hair was caused by exposure to the vapors of a very potent depilatory compound. Local protection of the scalp with caps or ointments did not prevent development of the alopecia. 5. No other local or general toxic symptoms were observed.

The most detailed study of the depilatory agents was made by Ritter and Carter (3) at DuPont and Co. Loss of hair among employees in the neoprene rubber plants at DuPont reached epidemic proportions during World War II. Therefore an investigation was started to eliminate the source of this industrial hazard. It soon was established that alopecia occurred only among workers engaged in the polymerization plants where chloroprene is converted to the synthetic rubber polymer, neoprene. In the course of this polymerization process, volatile cyclic compounds (dimers) are formed from condensation and cyclization of two molecules of chloroprene, according to the following scheme (5):



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Wh n workers were exposed to the volatile cyclic dimers of chloroprene, temporary hair loss resulted after a latency period of probably two weeks duration. Proper ventilation of the polymerization plants completely eliminated this hazard.

In order to study the depilatory compounds in the laboratory, Ritter and Carter prepared the dimers in large amounts, by refluxing chloroprene with an antioxidant in the absence of oxygen. The resulting mixture was then applied to the skin of dogs, cats, guinea pigs and mice. The hair of these animals fell out in 4–10 days and regrew shortly thereafter. It also has been claimed that the depilatory mixture removed the hair from dead animals, if applied within a few hours after death (3, 6).

The main difficulty encountered by Ritter and Carter in the preparation of the dimers was the inconsistency of the results obtained. Although the same method was used in most of their preparations of the intermediary polymers, the resulting compounds were sometimes quite inert, while at other times very potent depilatory substances were obtained. No reason could be found for these discrepancies. Experiments on human subjects were disappointing and assay methods of depilatory potency were not developed (6).

Having in mind the potential uses of these compounds in clinical and investigative dermatology, we undertook these studies of the depilatory agents with the following aims: 1. To develop a method which would result in consistently active depilatory compounds. 2. To investigate the pharmacologic and toxicologic properties of these compounds. 3. To study the mode of depilatory action in order to develop new compounds with a similar effect upon hair growth for eventual clinical application.

As the work progressed, we came to postulate that a certain chemical configuration was responsible for the hair loss. On this basis we studied the effect on hair growth of naturally occurring compounds, which were chemically related, such as vitamin A and squalene. Since squalene is a component of human sebum, the action of the latter upon hair growth was also investigated. The material therefore is presented in four sections: I. In vivo and in vitro studies of the cyclic dimers of chloroprene. II. New synthetic depilatory agents. III. The effect of vitamin A and squalene upon keratinization. IV. The depilatory action of human sebum.

I. STUDIES OF THE CYCLIC DIMERS OF CHLOROPRENE

Experimental

A. Preparation of the dimers

Chloroprene* was obtained in two forms: 1. Pure chloroprene, which must be stored in dry ice in order to prevent its polymerization. 2. A 50% mixture of chloroprene with xylene, containing 1% p-tertiary butyl catechol or phenothiazine as an antioxidant. Before use, xylene must be distilled over from this mixture.

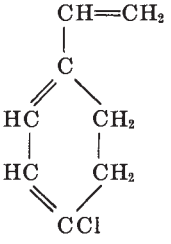
In order to prevent the rapid polymerization of chloroprene, 1% p-butyl-catechol or

* Obtained through courtesy of Albert S. Carter and Madison Hunt of E. I. DuPont de Nemours and Co.

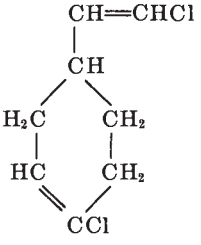
phenothiazine was added to the chloroprene. About 500 ml. or less of this solution was refluxed in a water bath over 4 days for a total of 44 to 48 hours. It was possible to interrupt refluxing overnight. In the course of refluxing the chloroprene polymerizes to compounds with a higher boiling point and the originally colorless or pale yellow solution alters to a dark brown oily mixture with a very strong terpene like odor.

As the refluxing continues, the mixture gradually thickens, (evidenced by the increasingly slow rise of the bubbles to the surface of the boiling mixture). At termination of the refluxing, the resulting mixture, consisting of unchanged chloroprene, intermediate compounds and higher, so-called ω-polymers, was cooled and half as much of pure methyl alcohol was added. This procedure precipitated the higher polymers in the form of a sticky, whitish, rubbery mass. The solution was left in the ice box overnight and the supernatant, consisting of chloroprene, intermediate polymers and methyl alcohol, was decanted. The precipitate was washed several times with methyl alcohol and the washings added to the decanted solution. The mixture was dehydrated by shaking with 10–20 Gm Na_2SO_4. Methyl alcohol and unchanged chloroprene were distilled over at a temperature below 80°, leaving the intermediary polymers behind. The dimer mixture was cooled, shaken with an equal amount of 10% aqueous Na_2CO_3 in a separatory funnel and left in the ice box overnight. The separated dimer was kept in the icebox in well-stoppered bottles. Seven batches of dimer mixture were prepared with this method, 5 with p-tertiary butyl catechol as an antioxidant, 2 with phenothiazine and p-tertiary butyl catechol. The yield was between 23 and 30%.

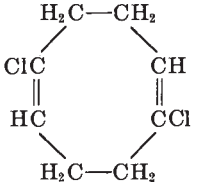
Through the courtesy of E.I. DuPont and Co. we also obtained two types of mixtures of dimers and the following purified intermediary polymers:

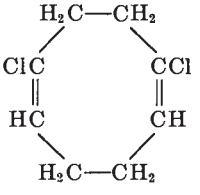


1-vinyl-4-chlorocyclohexadiene-1,3



1-α-chlorovinyl-4-chlorocyclohexene-3





Crude dichlorocyclooctadiene (a mixture of the two isomeric cyclooctadienes)

A higher polymer mixture, presumably containing polymers of more than two chloroprene molecules, i.e. compounds between the dimer and neoprene stage, was also tested.

B. In vivo studies

The dimer mixture was painted on the backs of albino mice (0.1–0.2 ml.), of mottled guinea pigs (0.5–2.0 ml.) and 12 day old chicks (0.2 ml. of 20% concentration). In rabbits, mostly the skin between the ears was painted, as this site was inaccessible to licking or scratching. In these animals the dimer was used in full strength or in a 5 to 50% dilution with 100% alcohol. In preliminary experiments the animals were treated every 2 or 3 days; later a single treatment was used routinely. No attempt was made at any point to remove

the hair mechanically or to test the firmness of its attachment by pulling. In our study, therefore, depilation means spontaneous loss of hair. A total of 41 applications were administered to the same number of mice, 15 to 15 guinea pigs and 53 local applications were given to 31 rabbits. Biopsy specimens and hair samples were taken from several animals of each species. In addition, specimens were taken from the back of a rabbit every other day after application of the dimer until the hair regrew, then again on the 28th and 60th day after treatment. The sections were stained with hematoxylin-eosin. Paraffin sections of normal rabbit skin and of rabbit skin excised on the first, 14th and 28th day after application of the dimer were also stained with the modified Bennett technique for sulfhydryl groups (7). All sections were left in the same sulfhydryl reagent for the same length of time.

Preliminary studies on the acute toxicity of the dimer were performed in rabbits with 2 ml. of 100% and 4 ml. of 25% dimer given per os. The subsequent more extensive acute toxicity studies were carried out in the laboratories of Smith, Kline and French. Oral doses of 1 ml. of 25, 35 and 50% concentrations of the dimer in ethyl alcohol were administered to three groups of ten rats (average weight 150 grams) respectively. Six control rats received 1 ml. of 95% ethyl alcohol or the maximum volume of alcohol administered with the dimer.

Percutaneous toxicity was tested on rabbits by daily application of the dimer to the area between the animals' ears. One ml. of 25, 50 or 100% concentration of the dimer was applied to each group of three rabbits.

We also performed chronic toxicity studies on a group of 14 albino mice of the C3H strain. Two tenths of one ml. of a 50% alcoholic solution of the compound was applied to the backs of the animals every 5 days for 5 months. A fresh batch of dimer was used at the end of every two months. A total of 30 applications was given. A control group of 7 mice was treated with 0.2 ml. of 100% alcohol.

Clinical trials on the scalps of children below the age of puberty were carried out with the crude mixture and with some of the individual purified dimers. One application of the crude mixture was given to 6 children; three children received 2 applications at weekly intervals; two children were treated 3 times at weekly intervals. Two children received a 50% ointment of the dimer mixture in Multibase^{R} (Ar-Ex) once daily for 4 days, one child a 40% mixture once daily in Neobase^{R} (Burroughs-Wellcome) for 3 days. Incorporation of the dimers in ointment bases greatly enhanced their irritating effect.

Crude dichlorocyclooctadiene incorporated in Neobase^{R} in a 17% concentration was administered to 2 children once daily for 5 days. A 10% preparation of 1-α-chlorovinyl-4 chlorocyclohexene-3 in a mixture of Neobase^{R} and alcohol was applied to a child once daily for 5 days. All children were observed during a follow-up period of at least 3 weeks' duration.

C. In vitro studies

The effect of the dimer on free sulfhydryl compounds in vitro was tested with Bennett's reagent (8).* In all these experiments 0.1 ml. of the dimer was added in serial dilutions with 100% alcohol, (ranging from 0.3% to full strength) to 0.1 ml. of buffered (0.1 ml. barbiturate buffer, pH 7.4) aqueous solutions or suspensions of sulfhydryl compounds. The following sulfhydryl compounds were tested: glutathione† (40–60 μg/0.1 ml.), 10% homogenates of mouse and guinea pig tissues and 20% homogenates of human epidermis, obtained by the heat method (9). The mixtures were subjected to frequent shaking for 15 minutes at room temperature before the determinations were carried out. Control solutions contained 0.1 ml. of 100% alcohol in place of the dimer.

Succinic dehydrogenase activity was measured in guinea pig and mouse tissue homo-

*Anson's method (11) could not be used because of interference by the dimer in the Prussian blue reaction.

† Obtained from Schwarz Laboratories, Inc., New York.

genates with the method of Kun and Abood (10) after incubating 1 ml. of 10% tissue homogenate with 0.1 ml. of 0.3–5% alcoholic solutions of the dimer at room temperature for 10 minutes. Control solutions contained 0.1 ml. 100% alcohol in place of the dimer. Only results obtained with 10% or lesser concentrations of the dimer were considered, because in higher concentrations the dimer, taken up by the acetone used in the method, gave rise to high colorimeter readings.

RESULTS

All seven batches of chloroprene dimers were about equally potent depilatory agents when tested on laboratory animals. A single application of the dimer mixture resulted in complete local loss of hair within 10–14 days. However, we were unable to obtain alopecia in 4 days, as described by Ritter and Carter (3). All batches had a more or less pronounced irritating effect on the skin, manifested by edema, erythema and infiltration. The thickened skin was cast off in the form of crusts to which the hair remained attached with hair roots protruding on the

FIG. 1. Hair loss in rabbit 14 days after single application of chloroprene dimer

dermal side (12). The underlying new skin was smooth and hairless. All eventual minor remnants of crusting and inflammation rapidly disappeared (Fig. 1).

The hair loss was not related to the irritating action of the dimers. Highly irritating batches were often less potent depilatory agents than those with a relatively small irritating effect.

It was necessary to devise assay methods to measure the depilatory potency of the dimers.* According to our experience, the most reliable method was the dilution test. In this test various alcoholic dilutions of freshly prepared dimer in 3.1 to 50% strength, were painted on various skin sites on the back of a rabbit. The minimum effective concentration which still caused complete hair loss was considered the measure of the depilatory effectiveness. In such tests, partial alopecia was obtained with 6.2% concentrations of freshly prepared dimers; 12.5% caused complete alopecia, while 3.1% was without effect. After two weeks the minimum depilatory concentration of the dimers for rabbits rose to 25 or

* The use of the length of the latency period as an index of depilatory potency, as suggested by Ritter and Carter (6), is open to question.

50%, while 4–6 weeks old batches did not cause complete depilation anymore in the rabbit. This deterioration occurred although the depilatory agents were kept constantly at ice box temperature. No method was found to prevent loss of depilatory potency of the agents or to restore their original effectiveness.

Of the animals tested, mice were the most sensitive to the dimer, guinea pigs much less and rabbits the least. Batches of dimers which no longer caused hair loss in rabbits, retained their depilatory action in guinea pigs. Even after becoming ineffective in guinea pigs, the dimers still were able to cause alopecia in mice. Loss of feathers was observed in chicks.

About 1 week after onset of alopecia, hair regrowth began and within a few weeks the fur was restored to normal. However, repeated application of the dimer led to irreversible thinning and coarsening of the hair in some of the animals tested.

In contrast to the findings of Ritter and Carter with dog experiments (3), we were unable to produce alopecia by oral administration of the dimer to rabbits or guinea pigs. When continuously exposed to the vapors of the dimer, guinea pigs lost no hair even after 4 weeks. Again, unlike Ritter and Carter, we were unable to produce postmortal depilation in mice. When, immediately following the death of the animal, mouse skin was soaked for several days in a mixture of saline and dimer, no loosening of the hair occurred. Nor did a strong current of water remove the hair as described on dogs (3).

When administered to rabbits per os, 2 ml. of the pure compound caused death within 3 days. Autopsy revealed severe gastric irritation, leading to perforation of the stomach. The diluted compounds were tolerated without any apparent ill effect.

Among the ten rats receiving 1 ml. of the 50% concentration by mouth, seven died within 72 hours. Four out of 10 died after 1 ml. of 35% solution, and 1 out of 10 after 1 ml. of the 25% concentration. Toxic symptoms included depression, dyspnea, prostration and piloerection. No toxic effects were observed in six control rats receiving 1 ml. of ethyl alcohol. From these studies it appears that 1 ml. of the 25% concentration of the dimer is the approximate minimal lethal dose.

Daily topical application of 1 ml. of 25%, 50% and 100% concentrations of the dimer to rabbits for one week caused skin irritation and local ulceration in most of the animals. Ten days after the beginning of the tests all of the rabbits showed a complete loss of hair in the treated area and small ulcerations in this region; however, these ulcerations disappeared by the 12th day.

Of the mice under treatment for 5 months, 4 died within the first week. At the end of 5 months six were alive; the others died of intercurrent infections. In the deceased animals no macroscopic or microscopic signs of malignancy could be found. The surviving mice remained bald throughout the entire period. Otherwise they exhibited no signs of ill health and gave birth to several normal litters. Even two months after cessation of treatment, the hair did not regrow completely in 4 out of 6 mice, but remained thinner on the treated area.

Experiments on human subjects led to negative results in all cases. The dimer proved to be strongly irritating and without any effect on the hair of the scalp.

When applied under a closed dressing for 24 hours these compounds caused erythema, later infiltration and extreme itching. These changes disappeared gradually in a few days with residual pigmentation.

Histologic studies on hair revealed marked hypoplasia of the hair bulb and lower part of the hair in all species tested. Fig. 2 shows a normal guinea pig hair with well-formed root and between hair bulb and medulla a "clear sector" only slightly thinner than the distal part of the hair. After treatment with the dimer the hair bulb shows signs of hypoplasia. A frequent finding is a small hook at the proximal end (6). The "clear sector" is often elongated and considerably thinned (Fig. 3).

Photographs of the histologic changes in the skin of rabbits have appeared in a previous publication (12). During the first few days after application of the dimer a severe inflam-

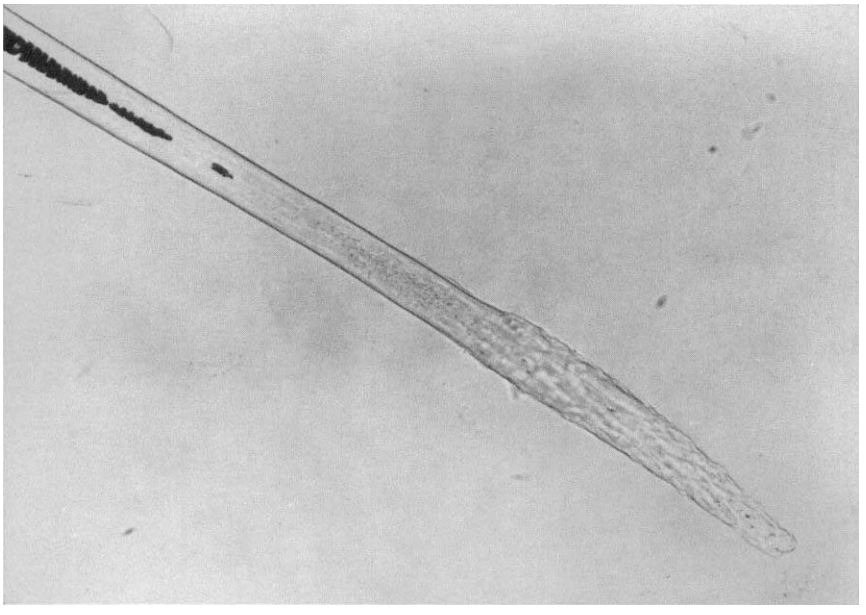


FIG. 2. Normal guinea pig hair

matory infiltrate could be found in the epidermis of all animals tested with degeneration of the polymorphonuclear leukocytes. The infiltrate was at no point in any relation to the hair follicles. Later (3rd to 7th day) the epidermis became necrotic, the sheath cells swollen and hyperplastic and the follicles contained much keratin. At the height of the alopecia, the epidermis of all animals tested was extremely thick and swollen and resembled human skin. There was hyperkeratosis extending into the dilated hair follicles. Most of the hair follicles disappeared; the deeply seated ones that remained were large and contained shrivelled keratinous material. The acanthosis persisted for 2–3 weeks. Four weeks after application of the dimer, numerous well-formed follicles could be seen again and 3 weeks later the skin resumed its normal appearance with thin epidermis, restored follicles and well formed hairs.

Similar changes were observed in the skin of rabbits, guinea pigs, mice and chicks. In all species the most conspicuous histologic features, (thickening of the epidermis, loss of hair and anomalies in keratinization) were reversible.

In vitro the dimer inactivated the free sulfhydryl groups of all the sulfhydryl

compounds tested to about the same extent. The percentage inactivation gave a linear relationship when plotted against μls of dimer (or percent of dimer in

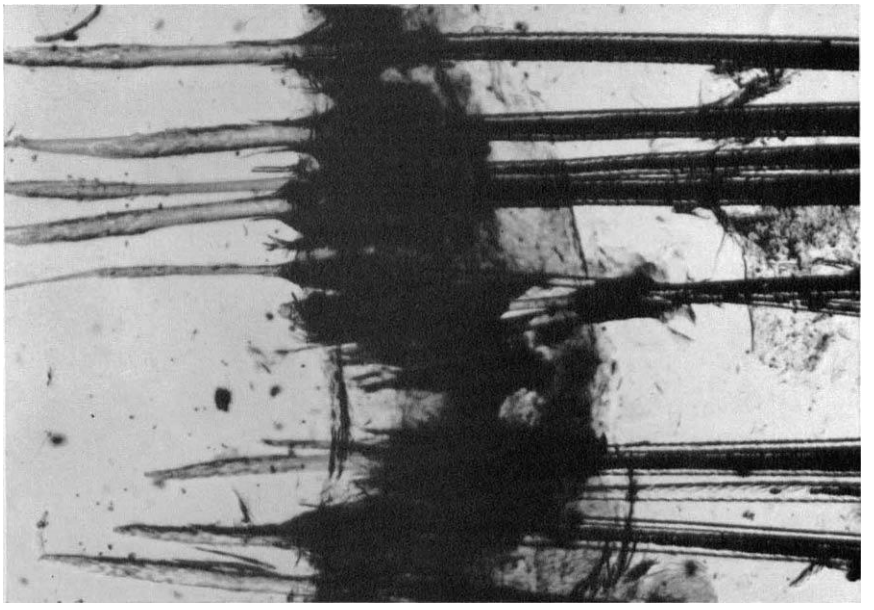


FIG. 3. Cast-off tuft of guinea pig hairs with crust after treatment with dimer. Note hypoplastic hair bulb.

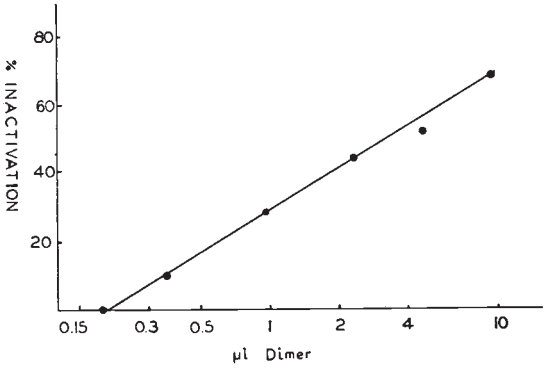


FIG. 4. In vitro inactivation of —SH groups of glutathione by dimer

0.1 ml. final volume) on semilogarithmic paper (Figs. 4 and 5). The *in vitro* inactivating concentrations corresponded very well with the *in vivo* depilating concentrations.* The inactivating effects of the dimer could not be eliminated

* The calculation is as follows: 1 ml. of 10% concentration of dimer caused alopecia over an approximate area of 16 sq. in. (100 cm. sq.) In previous experiments (14) we found that the weight of a sq. cm. of human epidermis is 3–7 mg. Assuming that the rabbit epidermis which is much thinner than human epidermis weighs 1 mg/cm. sq., 1 ml. of 10%

by *in vitro* pretreatment of the dimer with aqueous solutions of glutathione or cysteine. Inhibition of sulfhydryl was demonstrated also by histochemical methods (Figs. 6 and 7).

Succinic dehydrogenase activity in mouse and guinea pig liver was inhibited to the same extent to which the sulfhydryl groups were inactivated (13). This inactivation could not be counteracted by simultaneous addition of glutathione to the mixture or by pretreatment of the dimer with glutathione.

II. NEW SYNTHETIC DEPILATORY AGENTS

On the basis of our experiments with the dimer, the working theory was formulated that depilation was due to inactivation of sulfhydryl compounds in the skin. If this assumption was correct, the question arose what part of the dimer molecule could be held responsible for the sulfhydryl inactivation and for the

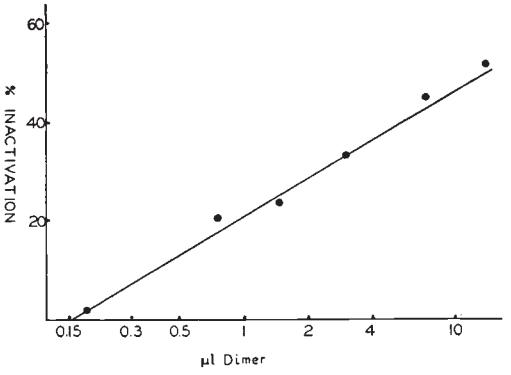


FIG. 5. In vitro inactivation of —SH groups of human epidermal homogenate by dimer

ensuing hair loss. It was suggested by Hunt (15) that the active grouping in the dimer molecule was the unsaturated double bond —CH=CH—. This group reacts with the free —SH groups according to the equation:

$$—CH=CH— + RSH \longrightarrow RS—\overset{1}{C}H—CH_2—$$

On this assumption we have tested to date 18 unsaturated compounds. Detailed data on these experiments have been published previously (16) and therefore only a summary of the findings will be reported here.

The compounds tested fulfilled the following requirements:

solution of the dimer (or 0.1 ml. of pure dimer) would cause depilation in the equivalent of 100 mg. of rabbit epidermis. In vitro 10 µl of the dimer inactivated 50% of the —SH groups of 20 mg. epidermis, or 100 µl (0.1 ml) inactivated 100 mg. which is the same value as obtained above. While these calculations are based on a number of assumptions which may not be valid (weight of rabbit epidermis, respective amounts of —SH in human and rabbit epidermis, neglect of the follicular epithelium in the calculations), the fact remains that the *range* in which the dimer is effective in vivo is close to the in vitro active concentrations.

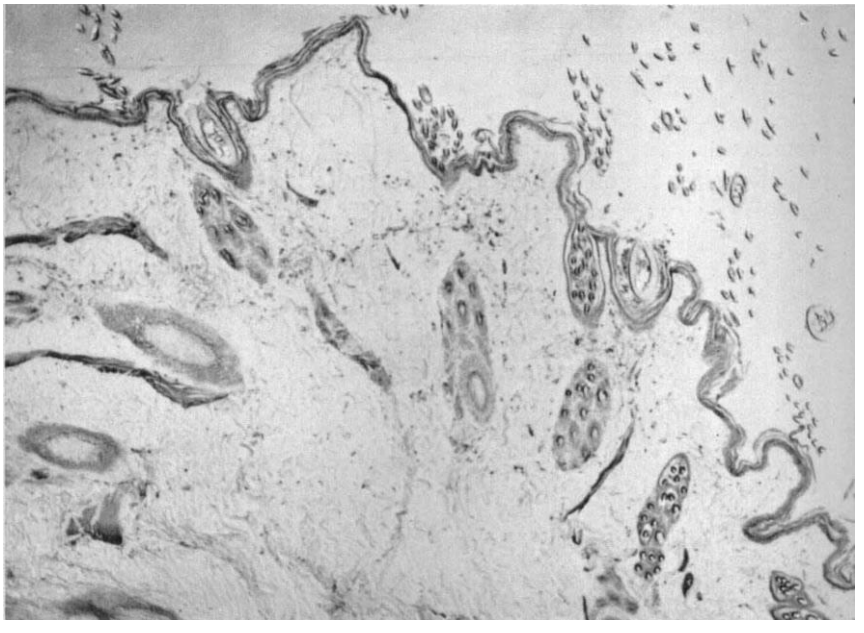


FIG. 6. Normal rabbit skin stained with Bennett's sulfhydryl reagent. Note dark epidermal band. 70× enlarged.

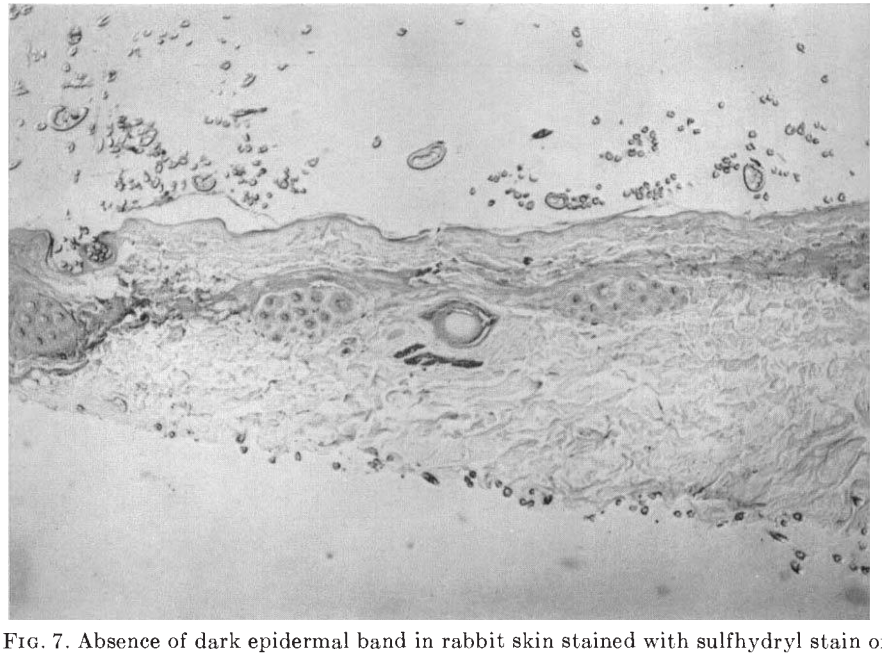


FIG. 7. Absence of dark epidermal band in rabbit skin stained with sulfhydryl stain one day after application of dimer in vivo. 70× enlarged.

1. All of them had the active grouping —CH=CH—.
2. None of the compounds contained Cl and thus did not give rise to the

HCl formation which had greatly contributed to the irritating effect of the dimer.

3. All of these compounds were of known composition, chemically relatively simple, pure and stable.

FIG. 8. Alopecia in mouse after local application of allyl laurate

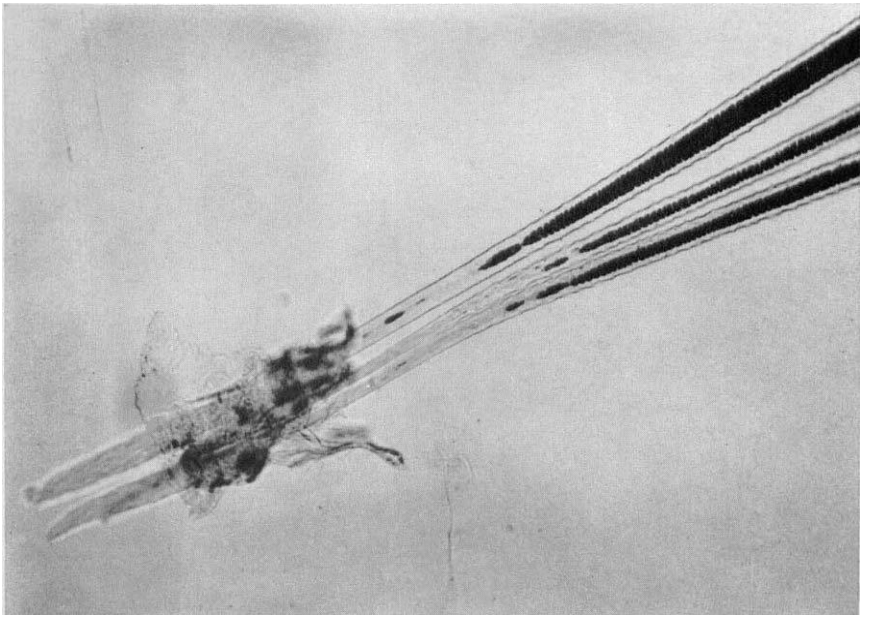


FIG. 9. Hypoplasia of guinea pig hair bulb after local treatment with allyl laurate

The unsaturated compounds were tested on mice, as this animal species proved to be most susceptible to the depilatory action of the dimer. Only after the compounds had been found capable of depilating mice, were guinea pigs and rabbits subjected to trial depilation. *In vivo* and *in vitro* studies were carried out in the same way as with the dimer. In addition to in vitro determinations of free —SH groups with Bennett's reagent (8), Anson's ferricyanide method (11) was also used, as, in contrast with the dimer, the new depilatory agents did not influence the ferri-ferrocyanide reaction.

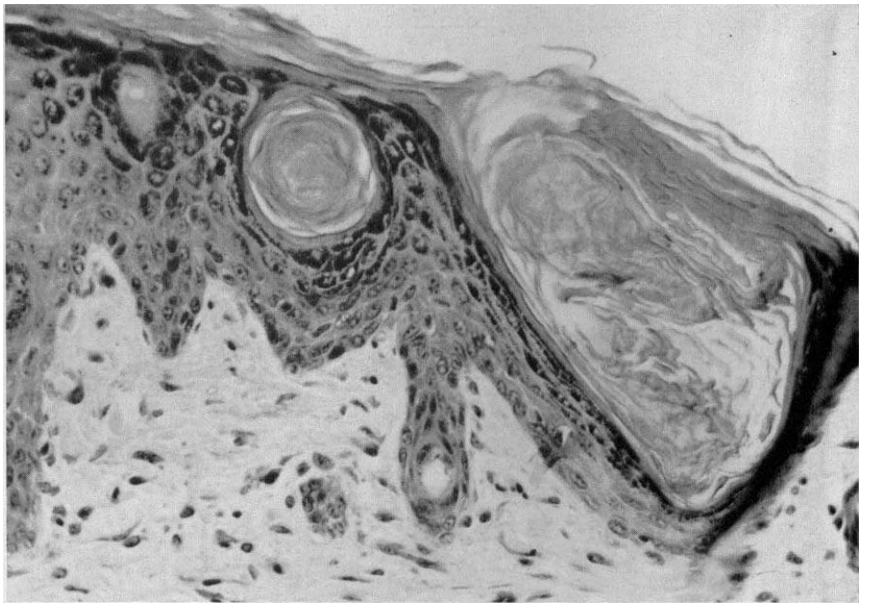


FIG. 10. Keratotic plugs in mouse skin after local treatment with allyl laurate. 400× enlarged.

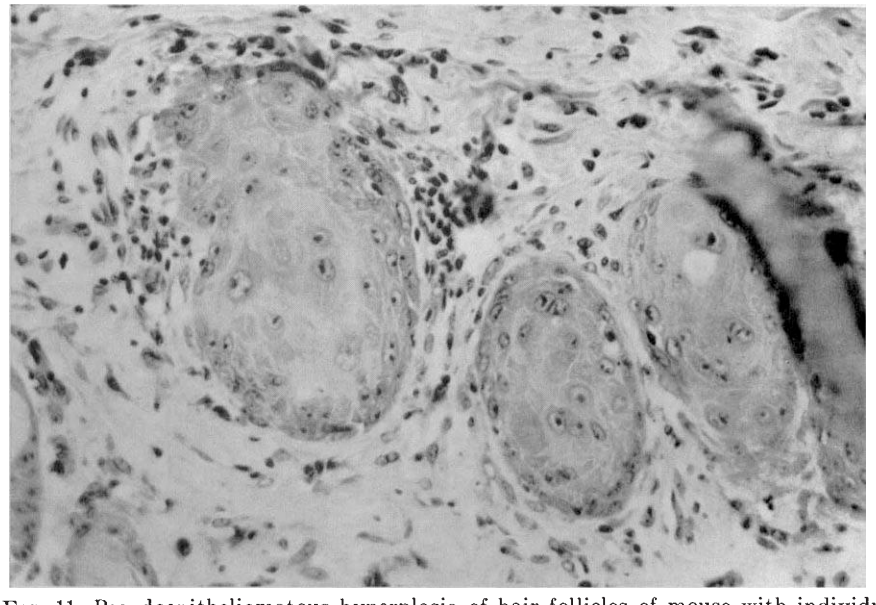


FIG. 11. Pseudoepitheliomatous hyperplasia of hair follicles of mouse with individual cell keratinization after local application of allyl laurate. 400× enlarged.

Three of the 18 compounds tested, namely allyl laurate, allyl benzoate and allyl diphenyl acetate, produced hair loss in mice (Fig. 8). The hair loss appeared as early as the 4th day after treatment with the depilatory agent, but otherwise followed a similar course as hair loss after application of the dimer. Allyl laurate

caused incomplete reversible hair loss in guinea pigs and allyl diphenyl acetate a slight degree of local baldness in rabbits.

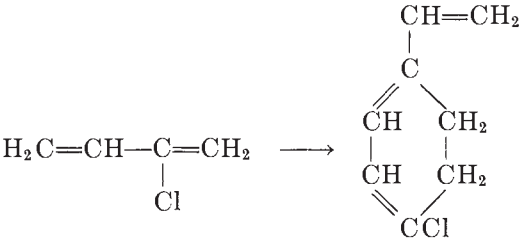
In preliminary tests the allyl compounds proved to be highly toxic. Mice were killed by 0.05 ml. applied to the skin; about half of the rabbits died within 24 hours from the percutaneous absorption of 1 ml. allyl benzoate. Guinea pigs were more resistant.

The allyl derivatives caused histologic changes in the hair (Fig. 9) and skin similar to those following application of the dimer. The epidermis became markedly thickened and the hairs were replaced by keratotic plugs (Fig. 10). The hair follicles showed signs of pseudoepitheliomatous hyperplasia with swollen epithelial cells, giant nuclei, individual cell keratinization and hypertrophic granular cells (Fig. 11).

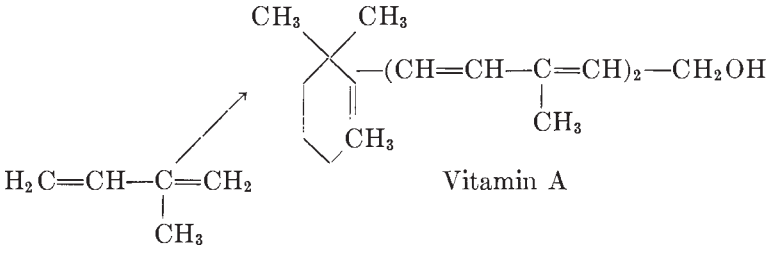
In vitro the allyl derivatives inactivated sulfhydryl groups to a much lesser extent than the dimer (16).

III. THE DEPILATORY EFFECT OF VITAMIN A AND OF SQUALENE

The close chemical relationship between vitamin A and squalene on the one hand, and of the dimer on the other hand, suggested that vitamin A and squalene might act upon keratinization by a mechanism similar to that of the dimer. Both vitamin A and squalene are polymers of isoprene, the parent molecule of natural rubber, whose sole difference from chloroprene lies in its having a CH_3 group in place of the Cl atom of chloroprene:

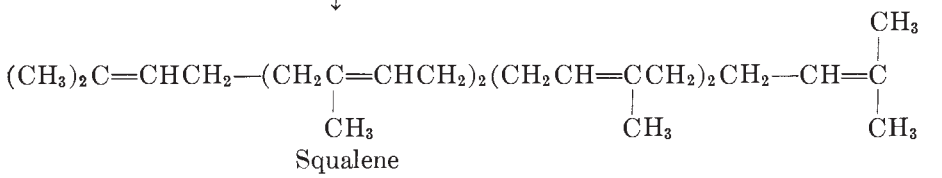


Chloroprene Dimer



Vitamin A

Isoprene



Squalene

Experiments on hair loss from locally applied natural (Fig. 12) and synthetic vitamin A have been described elsewhere (16). In vitro vitamin A had no effect on sulfhydryl compounds.

The depilatory action of squalene, a component of normal human sebum, has been described previously (17). The same results were obtained with two different batches of squalene (E. I. DuPont and Co., and Distillation Products Industries, Rochester, N. Y., "technical grade"). Vitamin A and squalene caused loss of hair without macroscopic or microscopic signs of inflammation.

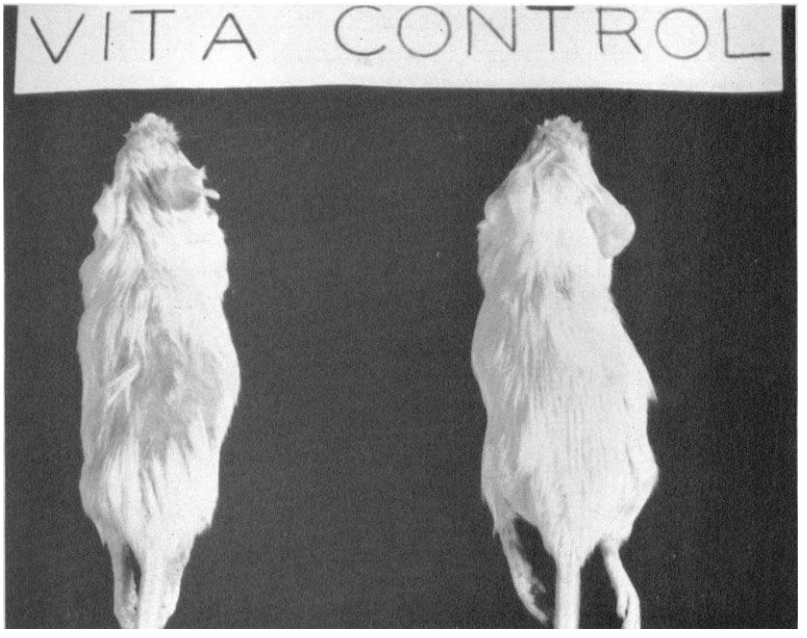


FIG. 12. Loss of hair in mouse after local application of natural vitamin A in corn-oil-alcohol mixture. Control animal treated with solvent only.

Therapeutic trials with squalene and vitamin A ointments in children with tinea capitis failed, in spite of the large doses administered (100,000 units of vitamin A palmitate/gm ointment and pure squalene applied freely) and the prolonged treatment (daily inunctions up to 4 weeks). Vitamin A was found to extinguish the fluorescence of hair infected with microsporon Audouini. Upon cessation of treatment the fluorescence returned.

IV. DEPILATORY ACTION OF HUMAN SEBUM

Since squalene is a component of sebum (18, 19), the effect of human hair fat on hair growth in laboratory animals was also investigated. "Sebum" was obtained by extracting the hair of male college students and of children (under the age of puberty) with ether. The ether extract was filtered and the ether evaporated at room temperature. The remaining honey-colored, semi-fluid sebum was

warmed to body temperature before it was painted without rubbing to the skin of rabbits, guinea pigs (1 ml. each) and of mice (0.2 ml.). Adult sebum was applied to 5 rabbits and 10 mice, children's sebum to 3 rabbits, 2 guinea pigs and

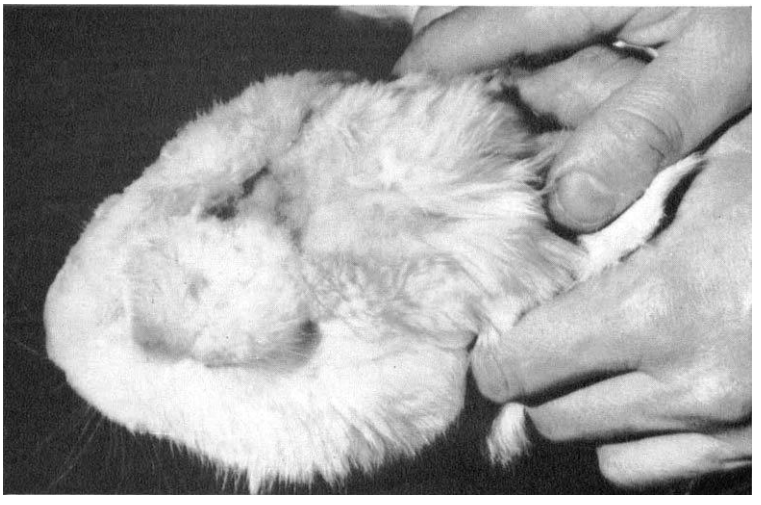


FIG. 13. Loss of hair in rabbit after single application of human "sebum" (hair fat)

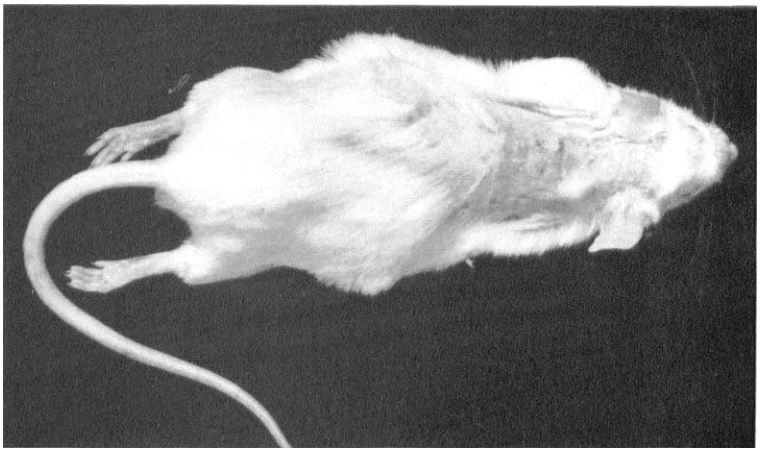


FIG. 14. Loss of hair in mouse after single application of human "sebum" (hair fat)

10 mice. Control groups of animals were treated with mineral oil and with 50% commercial lanolin in ether respectively. Experiments on the effect of sebum on sulfhydryl compounds *in vitro* were carried out with alcoholic dilutions of sebum ranging from 1:10 to 1:1000, in the same way as described above. *In vitro* experiments were also carried out with commercial lanolin, mineral oil and two samples of cerumen, in order to determine their effect on sulfhydryl compounds.

Ten to 12 days after a single application of sebum, all rabbits tested lost their hair at the site of treatment (Fig. 13). Only one of the mice developed extensive alopecia (Fig. 14); no baldness was noted on any of the other mice or on the guinea pigs.* There were no inflammatory changes. The hair loss was similar to that observed after treatment with squalene (17); the hairs fell out in small clusters which were embedded in non-exudative crusts. Histologic examination of the hairless skin of the mouse 2 weeks after treatment revealed hyperplasia of the sheath cells, especially of the fundal cells, with loss of the hair shafts. There were no inflammatory changes visible. The underlying skin was smooth and hairless. In every instance the baldness was reversible. Hair growth was resumed a week after the hair fell out and within a few weeks the fur was restored.

In vitro human sebum inactivated the sulfhydryl groups of glutathione and of mouse liver homogenates. The highest dilution at which a significant (10 to 20%) inhibition could still be observed was about 1:200. With increasing concentra-

TABLE I

	Synthetic series:	*Natural series:*
I. Mother substance:	$CH_2=C(Cl)-CH=CH_2$ Chloroprene	$CH_2=C(CH_3)-CH=CH_2$ Isoprene
II. Depilatory compounds:		
1. Dimer	(2 molecules of chloroprene)	1. Vitamin A (2 molecules of isoprene)
2. Higher polymers	(more than 2 chloroprenes)	2. Squalene (more than two isoprenes)
III. End product:	Synthetic rubber (Neoprene)	Natural rubber (Caoutchouc)

tions of sebum, the inactivation increased markedly. Alcoholic and ether solutions had about the same inhibitory potency. Sebum from children had essentially the same *in vivo* depilatory potency as sebum from adults. Lanolin and mineral oil had no effect on hair growth in rabbits, guinea pigs or mice and did not inactivate glutathione or succinic dehydrogenase *in vitro*. Cerumen had no effect on sulfhydryl groups *in vitro*. Detailed data on these experiments will be published in a forthcoming report.

DISCUSSION

There appears to be a certain analogy between the synthetic (chloroprene polymer) and naturally occurring (isoprene polymer) series of local depilatory agents (see Table I).

From our studies with these agents the theory has emerged that the depilatory action of these compounds is due to inactivation (alkylation) of epidermal

* In a recent series, 30% of the mice lost their hair when larger amounts (0.3–0.5 ml.) of sebum were applied.

sulfhydryl groups with a resulting interference in normal hair formation. This theory is supported by *in vitro* studies, histochemical observations and by the finding that the *in vivo* depilating and the in vitro sulfhydryl-inactivating concentrations of the dimer and of the allyl derivatives are in the same range. It must be emphasized, however, that a few *in vivo* inactive compounds proved to be highly effective sulfhydryl inhibiting agents *in vitro*. Obviously other factors, such as solubility of the depilatory compounds, modification of their chemical structures *in vivo*, etc. also influence the results obtained *in vivo*. At present the theory that interference with hair growth is due to sulfhydryl inactivation by the —C=C— bonds, rests on indirect evidence. Experiments providing more direct proof have been partly completed and will be published in a future report.*

The position of vitamin A is somewhat exceptional. The distribution of the —C=C— groups in the side-chain of this compound is not very favorable for sulfhydryl inhibition, as confirmed also by our *in vitro* studies. It is conceivable that not vitamin A itself, but one of its metabolic breakdown products causes the loss of hair. Hair loss from vitamin A has been described previously after administration of large amounts per os to animals (20–30) and to human subjects (31–34) and after local application to the skin of rats and porcupine (one experimental animal) (35).

We cannot offer any explanation for our failure to obtain depilation in human subjects with any of the agents tested. It is possible that the relatively thick human epidermis with the deeply seated follicles and the relatively long human hair cycle represent unfavorable conditions for the action of the depilatory compounds. In his studies with adrenal cortical hormones which cause local depilation in rats, but have no effect in man, Baker came to similar conclusions (36). However, the fact, that under certain conditions the chloroprene dimers and vitamin A cause loss of hair in human beings, raises the hope of developing a substance which could produce reversible localized baldness in man and would find clinical application in the treatment of fungous infections of the scalp.

From preliminary experiments it appears that the depilatory action of sebum cannot be attributed solely to its squalene content. In our experiments, human sebum had a several hundred times more pronounced inhibitory action on sulfhydryl compounds in vitro than could be accounted for on the basis of its squalene content and in a comparatively small experimental series we were unable to produce baldness in mice with squalene, while sebum depilated up to 30% of the animals tested.

It is impossible to assess the full implications of our finding that squalene and human sebum cause loss of hair in laboratory animals. This is the first instance in which a substance excreted onto the normal skin has been shown to influence growth of hair. It is therefore conceivable that this finding offers the first chemical clue to the etiology of common male baldness. The possibility that sebum plays

* Thus hydrogenation of squalene yields a compound, dodecahydrosqualene, with physical properties similar to those of squalene, but without unsaturated double bonds. Dodecahydrosqualene has no depilatory effectiveness and is inert as a sulfhydryl inhibitory compound in vitro.

an important role in the development of male baldness has been discussed by previous authors (37, 38). The essential role of male sex hormones in the development of this condition (39) is not contradicted by our findings, since male sex hormones are known to be powerful stimulants of sebaceous secretion. Our findings also introduce a new theory into dermatology: the concept, that sebum cannot be considered merely as an inert lubricating agent, but must be regarded as a chemically active mixture which influences the process of keratinization. Finally, the well-known carcinogenic effect of smegma (40) which has been attributed to the presence of squalene or squalene-like substances (18) must be reinvestigated in view of previous findings showing that one of the first effects of some chemical carcinogens in provoking cutaneous malignancy, consists of a combination between the carcinogenic compound and the free sulfhydryl groups of the epidermis (41). These possibilities will require further experimental investigation.

SUMMARY

1. A new class of compounds which cause reversible total hair loss when applied locally to laboratory animals, was studied *in vivo* and *in vitro*.

2. The first observed reversible loss of hair among workers in the synthetic neoprene rubber (chloroprene polymer) industry has been attributed to exposure to the vapors of the intermediary cyclic polymers (dimers). When these compounds were painted on mice, guinea pigs, rabbits and chicks, within 2 weeks reversible total alopecia followed at the site of application in all cases. The dimers were relatively non-toxic, rather unstable and irritating. The most conspicuous histologic changes were thickening of the epidermis, disappearance of the hair shafts and anomalies of keratinization.

3. In vitro the dimers inactivated free sulfhydryl compounds and the sulfhydryl enzyme succinic dehydrogenase. Good correlation was found between the *in vivo* depilating and sulfhydryl inactivating concentrations.

4. It was postulated that the depilatory action of these compounds was related to the sulfhydryl-inactivating effect and was due to the —C=C— groups in the molecules. Other unsaturated compounds with this grouping were tested and three of them, allyl laurate, allyl benzoate and allyl diphenyl acetate, were found to have similar *in vivo* and *vitro* actions as the chloroprene dimers. These new compounds were stable and less irritating, but more toxic and less potent than the dimers.

5. Vitamin A, an isoprene polymer with conjugated double bonds, was found to have a reversible local depilatory effect, similar to that of the other compounds studied. In vitro, vitamin A had no effect on sulfhydryl compounds.

6. Squalene, an unsaturated hydrocarbon (isoprene polymer), a natural component of human sebum, caused reversible hair loss in rabbits and guinea pigs without inflammation. In vitro, squalene inactivated sulfhydryl compounds and succinic dehydrogenase.

7. Human sebum, obtained by ether extraction of the hair of male students or of children, caused reversible hair loss in all rabbits and some of the mice

tested. In vitro sebum inactivated sulfhydryl groups and succinic dehydrogenase.

8. The possibility is raised that human sebum may play a role in the etiology of common male baldness. It is suggested that sebum should be considered as a chemically active mixture which may influence the metabolism of the skin.

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ADDENDUM

After completion of this manuscript, we found that oleic and linoleic acids, both unsaturated fatty acids, had a marked local depilatory action in rabbits and mice after a single application. In vitro these acids inactivated the sulfhydryl groups and succinic dehydrogenase activity of mouse liver in dilutions up to 1:100 and 1:200 These observations offer further proof in support of the theory advanced above. The finding that oleic acid caused loss of hair is definite evidence for the view that the depilatory action of human sebum is not solely or even chiefly due to its squalene content.

Our attention was also called to a recent case of hypervitaminosis A in an adult, described by Sulzberger and Lazar (J. A. M. A., **146:** 788, 1951). This patient had marked loss of hair; after discontinuation of the high vitamin A intake, the hair regrew completely.

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DISCUSSION

Dr. Weidman, *Philadelphia:* During the study of the sections from these animals, I was struck by the extreme grade of cellular hyperplasia in the bottoms of the hair follicles; on the basis of appearances in human beings it would classify as adenomatoid hyperplasia. Naturally, the thought occurred at once that perhaps we had in the depilatory agents a substance which induced cell growth and which might serve as a tool in experimental carcinogenesis. However, subsequent tests by Dr. Flesch in which the animals were sacrificed at different time periods, proved that this was not the case and that what we were observing was regeneration. It was, though, a regeneration of a most extraordinary kind, and I had no idea that regenerative hyperplasia could amount to anything like this in a hair follicle, at least as observed in man. Obviously, the mouse is a suitable test object for producing high grade cellular hyperplasia and depilatory compounds are agents with which to accomplish it.

The second thought that occurred to me arose when Dr. Pinkus read his paper on pili multigemini. You will recall the lantern slide showing on a large scale a transverse section of the hair follicle which contained four, or perhaps five transverse sections of hair shafts. Such a picture was reproduced in these mice; that is, the regenerative hyperplasia at the bottom of the follicles had taken place at multiple points,—not a single one, and had resulted in the formation of experimental, if you please, pili multigemini. In connection with this, and incidentally, the base of the hair follicle in mice does not exhibit definite papillae. The hair appears to develop from an ill-defined, non-circumscribed area of cells which constitutes the homologue of the papilla.

So I would state that an additional example of a cause for pili multigemini is apparent, to be added to the list which Dr. Pinkus constructed for us, namely, that they can occur in mice at least as an expression of regenerative hyperplasia.